ELSEVIER

Biosensors & Bioelectronics 14 (1999) 715–722

BIOSENSORS & BIOELECTRONICS

www.elsevier.com/locate/bios

Development of sensors for direct detection of organophosphates. Part II: sol–gel modified field effect transistor with immobilized organophosphate hydrolase

A.W. Flounders [a,*], A.K. Singh [a], J.V. Volponi [a], S.C. Carichner [a], K. Wally [a], A.S. Simonian [b], J.R. Wild [b], J.S. Schoeniger [a]

[a] *Sandia National Laboratories, Chemical and Radiation Detection Laboratory, PO Box 969, MS 9671 Livermore, CA 94551-0969, USA*
[b] *Texas A&M University, Department of Biochemistry and Biophysics, College Station, TX 77843-2128, USA*

Received 22 February 1999; received in revised form 9 August 1999; accepted 1 September 1999

Abstract

pH-sensitive field effect transistors (FET) were modified with organophosphate hydrolase (OPH) and used for direct detection of organophosphate compounds. OPH is the organophosphate degrading gene product isolated from *Pseudomonas diminuta*. OPH was selected as an alternative to acetylcholinesterase, which requires inhibition mode sensor operation, enzyme regeneration before reuse, long sample incubation times, and a constant source of acetylcholine substrate. OPH was covalently immobilized directly to the exposed silicon nitride gate insulator of the FET. Alternatively, silica microspheres of 20 or 200 nm were formed via a base catalyzed sol–gel process and were dip-coated onto the gate surface; enzyme was then covalently immobilized to this modified surface. All sensors were tested with paraoxon and displayed rapid response (<10 s), with a detection limit of approximately 1×10^{-6} molar. The 200 nm sol–gel gate modification enhanced the signal of enzyme-modified devices without effecting device pH sensitivity. Sensors were stored at 4°C in buffer and tested multiple times. Devices coated with 200 nm silica microspheres maintained significant enzymatic activity over a period of 10 weeks while uncoated devices lost all enzyme activity during the same period. The 20 nm sol–gel modification did not enhance device response or enzyme stability. Successful reuse of sensor chips was demonstrated after stripping inactive enzyme with an RF oxygen plasma system and reimmobilizing active enzyme. Published by Elsevier Science S.A.

Keywords: Organophosphate; Sensor; Organophosphate hydrolase; Acetylcholinesterase; Enzyme; Immobilization; Sol–gel; Field effect transistors; ISFET; pHFET; EnFET; Acetylcholinesterase; Organophosphate hydrolase

1. Introduction

A wide variety of sensors have been proposed and investigated for organophosphate detection. Enzyme (Dumschat et al., 1991), antibody (Van Emon and Lopez-Avila, 1992; Makower et al., 1997) and whole cell (Dutka et al., 1983) recognition elements have been investigated and piezoelectric (Guilbault et al., 1981/82), optical (Rogers et al., 1991; Pandey and Weetall, 1995) and electrochemical (Trojanowicz and Hitchman, 1996) transduction platforms have all been developed. Recognizing that organophosphate poisoning is due to highly irreversible binding to and inactivation of acetylcholinesterase (AChE), the most prevalent biosensor strategy has been enzyme-based sensors that mimic this organophosphate mode of action. These sensors use acetylcholinesterase or butyrylcholinesterase as the sensor recognition component while the sensor transduction element is a colorometric or electrochemical platform for monitoring cholinesterase activity. Cholinesterase-based sensors for organophosphate are inherently inhibition mode. Organophosphate capture results in loss of enzyme activity and hence a decrease in sensor signal. Acetylcholinesterase-based sensors suffer from several limitations. First, any environmental or handling factors that may cause loss of cholinesterase activity may result in false positive signal. Second,

* Corresponding author. Tel.: +1-925-2941367; fax: +1-925-2941489.
E-mail address: awfloun@sandia.gov (A.W. Flounders)

0956-5663/99/$ - see front matter Published by Elsevier Science S.A.
PII: S0956-5663(99)00045-7

AChE-based sensors require baseline testing prior to sample application and lengthy sample incubation times to allow AChE-analyte interaction. Third, due to the irreversible nature of acetylcholinesterase inhibition, AChE sensors can not be reused without incubation in a cholinesterase regenerating solution such as pyridine-2-aldoxime methiodide.

In order to take advantage of the demonstrated high sensitivity and specificity of biomolecular recognition, yet avoiding the inhibition mode of cholinesterase-based sensors, we have investigated use of an organophosphate hydrolase enzyme for direct detection of organophosphate compounds. Organophosphate acid anhydrases are a broad class of enzymes of great interest (Reiner et al., 1989) due to their ability to hydrolyze organophosphate compounds into much less toxic products. These enzymes were initially separated into two major categories, Mazur type and squid type (Hoskin et al., 1984), based upon enzyme source, substrate preference and a variety of enzyme sensitivities. Various enzyme extracts have also been described as DFPases, paraoxonases, parathionases etc. emphasizing the substrate preference of each. However, these distinctions have become less meaningful as multiple components have been identified in some enzyme extracts, additional enzymes have been isolated from other sources, and attempts to categorize the enzymes based upon consideration of phylogenetic relationships have been emphasized (Noellgen and Landis, 1992). An overview with some focus on this nomenclature debate has been presented by one of the pioneering investigators of these enzymes (Aldridge, 1989). Following the recommendation of Aldridge et al. (1989) and the example of Dumas et al. (1989), we use the term organophosphate hydrolyzing enzyme as a general term describing those enzymes which catalyze hydrolysis of numerous organophosphate triesters and organophosphofluoridates.

The most well characterized of these enzymes is the organophosphorous degrading (opd) gene product of *Pseudomonas diminuta*, which we describe here as organophosphate hydrolase (OPH). Purification and organophosphate degrading capability (Dumas et al., 1989, 1990), sequence information (McDaniel et al., 1988), catalytic mechanism (Lewis et al., 1988), crystal structure (Benning et al., 1994) and folding mechanism (Grimsley et al., 1997) have been reported. In addition, enzyme expression in non-native systems (Phillips et al., 1990; Dave et al., 1994), site directed mutagenesis (Lai et al., 1994, 1996; Watkins et al., 1997)) and enzyme encapsulation (Pei et al., 1994) have also been investigated. The enzyme has been expressed in *E. coli* cells and these cells were cryoimmobilized and used for organophosphate detection in a continuous flow reactor system. Micromolar detection and excellent sensor stability were reported (Rainina et al., 1996). Recently, immobilized OPH was combined with an amperometric AChE-based biosensor for discriminative determination of carbamate and organophosphate pesticides (Simonian et al., 1997).

We have extended use of OPH from *P. diminuta* in a sensor system by immobilizing OPH to the gate of a pH-sensitive field effect transistor (FET). Enzyme-modified FETs have been proposed and investigated for many analytes. The acetylcholinesterase-modified FET for organophosphate detection was a direct extension of cholinesterase-modified electrodes (Strop and Guilbault, 1972) and was proposed (Janata et al., 1981) soon after the initial enzyme-modified FET reports (Caras and Janata, 1980). Investigation of cholinesterase-modified FETs has continued (Dumschat et al., 1991; Vlasov et al., 1991; Sakai et al., 1993; Nyamsi Hendji et al., 1993), and the same strategy (inhibition mode/potentiometric detection) has been pursued with a commercially available, light addressable potentiometric sensor (Fernando et al., 1993). However, we believe this report is the first use of the organophosphate hydrolase modified field effect transistor for organophosphate direct detection. Results indicate that the OPH-modified FET is suitable for detection down to micromolar concentrations of paraoxon in less than 10 s.

There is a significant literature related to pH-sensitive FETs (see, for example, Janata and Huber, 1985; Bergveld and Sibbald, 1988). Early investigations were frustrated by electrically leaky silicon dioxide and silicon nitride and physically leaky encapsulation. More recent reports and the commercial availability of FET-based pH sensors indicate that many of these material issues have been resolved. An excellent historical review of the evolution of the device has been presented (Janata, 1994). A better understanding of device response also led to creative and enhanced dynamic signal measurement techniques (Van Der Schoot and Bergveld, 1987, 1990). With more stable and reliable devices available, efforts have focused on resolving long recognized enzyme-modified FET issues: influence of solution buffer capacity (Shul'ga et al., 1993), reference electrode miniaturization (Lisdat and Moritz 1993), and enzyme loading and stability.

In addition to the use of a novel enzyme, we have attempted to enhance the enzyme immobilization platform to improve enzyme loading and stability by physically modifying the gate insulator surface. Prior work (Singh et al., 1999) indicated that immobilization to porous silica beads increased enzyme stability and total activity relative to immobilization to non-porous surfaces, and a silica microsphere-modified surface is physically similar to porous silica beads. Therefore, silica microspheres of 20 or 200 nm diameter were formed via a base catalyzed sol–gel process, then dip-coated onto the FET gates. The same strategy has been used to

modify platinum electrodes and enhance stability of a glucose oxidase based enzyme electrode (Yang et al., 1995). Results indicate that the silica microsphere-modified gate did not affect device pH sensitivity and did enhance sensor performance indicating an increase in total enzyme activity. Results also indicate that sol–gel coatings were effective at improving enzyme stability. Sol–gel-coated devices with immobilized enzyme still required refrigerated storage in buffer.

2. Methods and materials

2.1. Sensor chips and control circuit

Sensor chips were prototype pH-sensitive FETs (SenDx Medical, Inc., Carlsbad, CA). Each chip contained two discrete depletion mode, n-channel transistors with a non-metallized gate insulator stack of thermal silicon dioxide and chemical vapor deposited (CVD) silicon nitride. A slightly serpentine gate geometry similar to that used by Shul'ga et al. (1993) was used. Devices were encapsulated at the wafer scale with polyimide (Dupont PI2555). Wafers were diced (Disco DAD2H, Santa Clara, CA), then chips were mounted into pin grid array (PGA) packages and wire bonded with a thermal ultrasonic wedge–wedge bonder (K&S, Inc. Santa Clara, CA). Bond wires were encapsulated with two component epoxy (Master Bond EP21ND, Hackensack, NJ). We found chip encapsulation with Shell Epon 825/Jeffamine D230 epoxy (Janata and Huber, 1985) unable to withstand long term aqueous saline buffer exposure. Epoxy encapsulation defined isolated liquid wells (~ 5 μl) around each device.

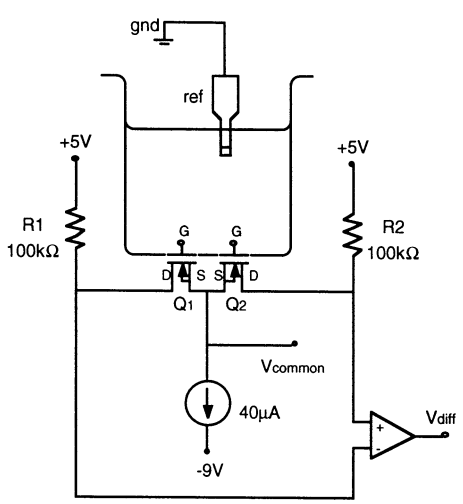

Fig. 1. Schematic of the constant current differential sensor circuit. Enzyme is immobilized to the gate of device Q1 while device Q2 serves as a non-enzyme coated reference. Bulk pH changes are measured as changes in the common source voltage V_{cs} and enzyme specific responses are measured as changes in V_{diff}.

A simplified schematic of the control circuit used to monitor the enzyme-modified and non-enzyme-modified transistor pair is shown in Fig. 1. The circuit is configured as a classical differential pair amplifier. Similar differential constant current control systems have been investigated (Tahara et al., 1982). The main advantage of the differential pair is that common mode variations such as temperature and bulk pH changes are eliminated while local pH changes at the immobilized enzyme FET gate are amplified. This differential approach has been well documented (Wong and White, 1989; Perrot et al., 1989; Rocher et al., 1994).

2.2. Sol–gel modification and enzyme immobilization

Silica microspheres were formed from tetraethoxysilane (Gelest, Inc., Tullytown, PA) via a base catalyzed sol–gel process then applied to diced chips by dip coating as described previously (Singh et al., 1999) with the following modifications. No plasma cleaning was performed for these samples so that effect of plasma treatment could be independently determined and firing temperature was limited to 300°C to minimize polyimide degradation. Dip coating was performed with diced chips prior to packaging, wire bonding and epoxy encapsulation. Sol–gel sphere diameter was determined with a sub-micron particle analyzer (Coulter, Model N4MD) and verified with electron microscopy. Spheres were deposited over the entire chip surface. Spheres were scraped with tweezers from metal bond pads prior to wire bonding; spheres not in the gate region were completely covered with epoxy encapsulation. Sol–gel and non-sol–gel coated chips underwent identical enzyme immobilization and paraoxon testing procedures.

Organophosphate hydrolase (EC 3.1.8.1) from a recombinant *E. coli* strain was isolated and purified according to methods previously described (Lai et al., 1994). Enzyme was immobilized to packaged chips following the aqueous aminopropyltriethoxysilane (APTS)/glutaraldehyde covalent attachment strategy described previously (Singh et al., 1999). The APTS/glutaraldehyde was the preferred immobilization chemistry since it yielded higher enzyme total activity in prior bead and glass slide studies. Sensor chips were treated with 1 N HCl, 15 min followed by 30% H_2O_2, 30 min for surface cleaning and silanol activation rather than the sulfuric acid, hydrogen peroxide activation used previously (Singh et al., 1999) to limit encapsulant degradation. Silane and glutaraldehyde treatments were performed by immersing packaged chips in solution. Enzyme was applied to only one device gate by pipetting enzyme into only one epoxy defined well. After extensive rinsing, chips were stored at 4°C in 10 mM phosphate buffered saline (pH 7.4). Some packaged chips were reused by stripping enzyme in an oxygen plasma reactor (March Instruments, PX1000) at 50 W,

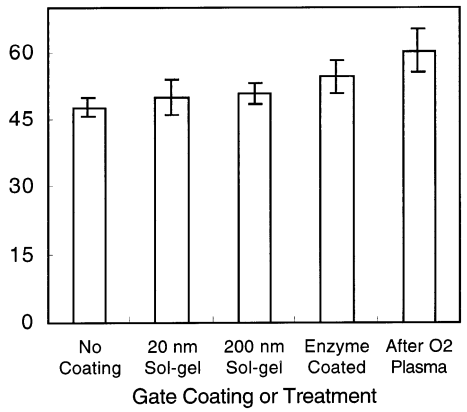

Fig. 2. Comparison of pH response after gate treatments. No Coating: no sol–gel coating and no chemical treatments; 20 nm sol–gel: coated with 20 nm sol–gel spheres no additional treatments; 200 nm sol–gel: coated with 200 nm sol–gel spheres no additional treatments. Enzyme coated: no sol–gel coating and after enzyme immobilization procedure. After O_2 Plasma: no sol–gel coating and after enzyme immobilization and 5 min, 50 W, 300 mtorr, 100 sccm O_2, plasma treatment. Sensors tested at pH 4, 7 and 10. $N = 4$.

300 mtorr, 100 sccm O_2, 5 min. Chips were retested for pH response after oxygen plasma stripping and then recoated with enzyme by repeating all immobilization chemistry treatments.

2.3. Testing procedure

All chips were soaked in deionized water for at least 24 h prior to testing. Devices were tested either (a) in batch mode by mounting a sealing ring over the encapsulated chip package which created a solution well (Fig. 1) of approximately 1.2 ml volume; or (b) in a flow system consisting of a peristaltic pump and a flow cell which mounted over the PGA package providing a head space of approximately 75 μl. A silver/silver chloride reference electrode was immersed either in the test solution (batch) or in the waste outlet stream (flow). Measurements were taken only with flow stopped to avoid streaming potential interferences. Device pH response was tested by monitoring changes in the common source voltage (V_{cs}, Fig. 1) with pH 4, 7, and 10 standard buffers (Sigma, St Louis, MO) before and after enzyme immobilization, sol–gel coating, and oxygen plasma treatment. Though pH 4 and 10 are not extreme conditions for enzyme exposure, chips with immobilized enzyme that were used for pH testing were not used for paraoxon testing unless fresh enzyme was immobilized to the chips. Chips were tested for organophosphate sensitivity in glycine–NaOH buffer (0.5, 1, or 10 mM with 0, 50, 100 mM NaCl) at pH 9.0 by adding varying volumes of paraoxon also diluted in glycine at pH 9.0 and monitoring changes in differential output voltage (V_{diff}, Fig. 1). The rate of hydrolysis of paraoxon in the absence of OPH was measured independently spectrophotometrically. This non-enzymatic hydrolysis was non-zero, but it was determined to be non-critical relative to the time scale of all sensor measurements. Independent measurement of bulk pH after addition of paraoxon to the sensor solution was performed by removing a sample from the sensor solution and testing with the same pH meter used for all buffer testing and adjustments (Orion 601, Beverly, MA).

3. Results and discussion

Fig. 2 compares the pH response (average of four different sensors) for a variety of gate treatments. Response has been averaged over the entire pH range tested (pH 4–10). We did observe slightly greater sensor response in the pH range 7–10 than in the pH range 4–7 as reported previously (Bergveld and Sibbald, 1988) and attributed to the more slowly varying potential of the silicon nitride surface closer to its point of zero charge. No significant difference in pH sensitivity was recorded for chips that underwent sol–gel modification or enzyme immobilization (Fig. 2) or both (data not shown). Change in pH response after chemical treatments has been reported (Bataillard et al., 1987; Bergveld and Sibbald, 1988). Therefore, it was important to verify that the enzyme immobilization or sol–gel modification did not result in a significant loss in device pH sensitivity.

A significant ($P < 0.05$) change in sensor pH response was noted after oxygen plasma treatment (Fig. 2). We believe this is the first report of the effect of oxygen plasma treatments on exposed gate field effect transistor pH response. Increase in surface silanol density after oxygen plasma treatment is expected (Ratner et al., 1990) and may explain this result. However, it has also been proposed that oxidation of the silicon nitride gate surface is responsible for a gradual decrease in pH response. Recovery of pH response by removal of oxidized nitride with hydrofluoric acid (Chen et al., 1989) or nitridation of surface oxide using rapid thermal processing has supported this hypothesis (Garde et al., 1995, 1997). It was surprising then that intentional oxidation via oxygen plasma treatment resulted in a pH sensitivity increase. A possible explanation is that all treatments (HF, RTP, O_2 plasma) succeed in removing adsorbed contaminants and therefore increase silanol or amine density and resulting pH response. It should be noted that devices were soaked in water for at least 24 h after plasma treatment and before pH response was measured (Diot et al., 1985) to insure full hydration of the pH-sensitive silicon nitride. After the sensor was coated with fresh enzyme, pH sensitivity returned to the lower 'Enzyme Coated' value presented in Fig. 2. We did not determine whether the pH sensitivity increase of

oxygen plasma treated sensors was maintained over time; such investigations accompanied by appropriate surface analysis appear warranted.

Sensor response to paraoxon concentrations from 10 to 50 μM (batch) and from 0.5 to 50 μM (flow) is presented in Fig. 3a. The batch data represent the averaged response of several different chips and the flow data represent the averaged response of multiple tests with the same chip. With the batch system, reproducible measurements were possible for paraoxon concentrations as low as 5 μM, while the flow system enabled reproducible measurements to 0.5–1 μM paraoxon. It should be emphasized that the two systems were set up at different laboratories and used by different experimenters; agreement between the two systems was excellent. To demonstrate the time scale of response, sensor signal during repeated injections of paraoxon to the flow cell is presented in Fig. 3b.

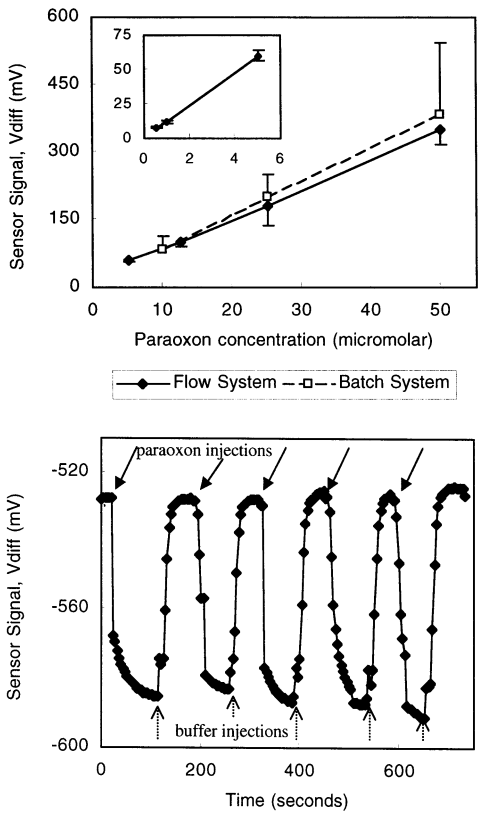

Fig. 3. (a) Non sol–gel coated sensor response to paraoxon. All tests conducted in 1mM glycine-NaOH buffer pH 9.0. Inset shows flow system response to concentrations below 10 μM paraoxon. Flow system, $N = 5$; batch system, $N = 6$. (b) Non sol–gel coated sensor response to paraoxon. All tests conducted in 1 mM glycine-NaOH buffer pH 9.0. 5 μM paraoxon injected at each solid arrow, glycine buffer injected at each dashed arrow. Time interval between data points is 4 s.

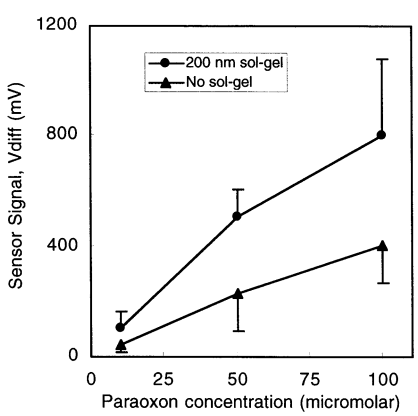

Fig. 4. Comparison of sensors with and without sol–gel coatings. All tests conducted in 1 mM glycine-NaOH buffer pH 9.0. $N = 4$.

A comparison of signal from sensors with and without sol–gel coatings to 10–100 μM paraoxon is presented in Fig. 4. Device response was enhanced with the 200 nm sol–gel coating. Since the sol–gel coatings did not affect pH sensitivity (Fig. 2), the measured signal increase is most likely due to an increase in enzyme loading or immobilized enzyme specific activity. This result correlates well with our earlier work demonstrating that the total activity of OPH is enhanced by immobilizing to porous rather than non-porous particles (Singh et al., 1999).

Sensor response with the 20 nm sol–gel modification was the same as that of uncoated sensors. Assuming closest packed spheres, the pore size provided by the 20 nm sol–gel coating is one third of particle diameter or approximately 67 Å. Unit cell dimensions of single crystal enzyme from X-ray diffraction are 80.3, 93.4 and 44.8 Å (Benning et al., 1994). This may explain why the 20 nm coating had no effect on sensor response; enzyme was unable to penetrate a silica microsphere matrix with such a small pore size. The much larger pores provided by the 200 nm sol–gel coating would not limit enzyme penetration, and an increase in immobilized enzyme loading is expected. The 200 nm sol–gel coated sensors still displayed widely varying chip to chip response (CV = 25–30%). In summary, the sol–gel coatings may have increased sensor response by increasing immobilized enzyme total activity; however, on a single chip surface, the sol–gel coating does not increase the surface area to volume ratio to such an extent that planar variations in enzyme loading are removed. With such a small area for direct immobilization, highly controllable and reproducible enzyme loading and activity continues to be a concern and the most significant source of sensor variability.

Our devices displayed the well-recognized (Caras and Janata, 1985; Eddowes, 1985) strong influence of solution buffer capacity on device response (Fig. 5). It was suspected that the sol–gel coatings may either act as a membrane and diminish the effect of buffer strength (Shul'ga et al. 1993) or act as an additional solid state buffer and further diminish device response. However, no difference was noted between devices with and without sol–gel coatings with respect to buffer strength.

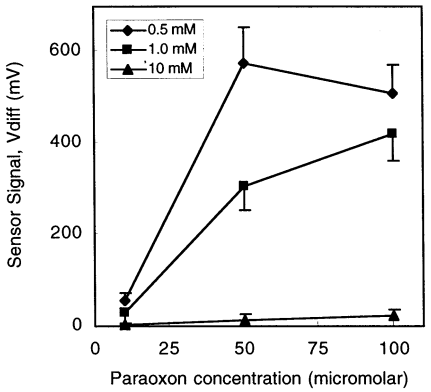

Fig. 5. Effect of buffer strength on sensor response. No sol–gel coatings. All tests conducted in glycine-NaOH buffer pH 9.0. $N = 3$.

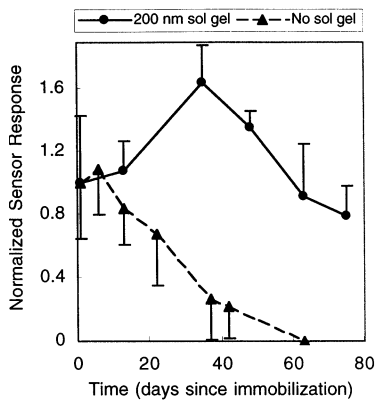

Fig. 6. Sensor signal versus time since enzyme immobilization. All tests conducted in 1 mM glycine-NaOH buffer pH 9.0. Sensor response to 10, 50 and 100 mM paraoxon normalized with respect to sensor response on first day after immobilization. $N = 3$.

For batch type experiments, buffer strength of 1 mM was selected as a compromise between satisfactory signal and the ability of an open system (subject to CO_2 absorption) to maintain constant pH. It should be noted that the independent pH measurements performed on removed samples indicated no measurable change in bulk pH. All buffer strengths tested were sufficient to compensate the acidic product generated by the immobilized enzyme once it diffused into the bulk. The sol–gel coatings in no way increase enzyme density to the point that dramatic changes in bulk pH are recorded.

Devices with and without sol–gel coatings were tested with paraoxon at 1–2 week intervals over a 10-week period (Fig. 6). Devices were stored in PBS at 4°C between measurements. The 200 nm sol–gel-modified devices maintained greater than 80% of initial activity for the entire 10-week test period. The 20 nm sol–gel coated and uncoated devices gradually lost all activity during the same period. This result supports our prior work (Singh et al., 1999) which demonstrated that porous immobilization platforms are superior to non-porous immobilization platforms at maintaining immobilized enzyme activity. Again, we attribute the poor performance of the 20 nm coatings to the pore size being too small for enzyme intercalation. A similar increase in enzyme stability with a preference for specific silica microsphere diameter has been reported (Yang et al., 1995). All devices lost all activity after day 75; failure to include any preservative in the storage buffer may have been responsible for the sudden loss of activity of the 200 nm sol–gel coated devices. Additional tests are being performed to determine the maximum sensor lifetime extension provided by the sol–gel coatings and to investigate the increase in enzyme activity noted in both the bead and sensor studies.

4. Conclusions

The OPH enzyme from *P. diminuta*, extensively investigated and characterized for its ability to hydrolyze organophosphate compounds, is an excellent candidate for biospecific direct detection of organophosphates. Results indicate that immobilization of OPH enzyme to the exposed gate of a pH-sensitive field effect transistor yields an organophosphate sensor with rapid response to micromolar concentrations of paraoxon. Additional investigations with other organophosphate compounds are underway. Though the sensor does not provide the nanomolar organophosphate detection limits exhibited by acetylcholinesterase-modified FETs, OPH-modified FETs do not require a continuous supply of consumable enzyme substrate, have a much faster response time, can be reused, and are far less likely to generate false positive signal.

The commercial availability of FET-based pH sensors indicates that many of the early material problems of these devices have been resolved. Enzyme-modified FETs will reach an equivalent degree of maturity only by addressing fundamental issues related to enzyme immobilization and stability. The sol–gel gate modifications investigated appear to provide a gate surface more suitable for enzyme immobilization. The 200 nm sol–gel coatings increased enzyme loading by increasing available surface area and enhanced enzyme stability. It should be stressed that this surface modification is not a mechanism for enhancing pH sensitivity or diminishing the inherent dependence of the enzyme-modified FET on the buffer capacity of the surrounding electrolyte. However, due to its inherent miniaturization, low power consumption, and ease of integration, the FET is still an extremely appealing transduction platform. With further improvements in reproducibility of enzyme loading and stability of enzyme activity, the enzyme-modified FET is an excellent sensor platform in those cases where the detection needs correspond to the limits imposed by enzyme catalytic parameters and system buffer capacity.

References

Aldridge, W.N., 1989. A-esterases and B-esterases in perspective. In: Reiner, E., Aldridge, W.N., Hoskin, F.C.G. (Eds.), Enzymes Hydrolyzing Organophosphorus Compounds. Ellis Horwood, Chichester, England.

Aldridge, W.N., Hoskin, F.C.G., Reiner, E., Walker, C.H., 1989. Suggestions for a nomenclature and classification of enzymes hydrolyzing organophosphorus compounds. In: Reiner, E., Aldridge, W.N., Hoskin, F.C.G. (Eds.), Enzymes Hydrolyzing Organophosphorus Compounds. Ellis Horwood, Chichester, England.

Bataillard, P., Clechet, P., Jaffrezic-Renault, N., Kong, X.G., 1987. The preparation of chem FET selective gates by thin silic layer grafting and their behavior. Sens. Act. 12, 245–254.

Bergveld, P., Sibbald, A., 1988. Analytical and Biomedical Applications of Ion Sensitive Field Effect Transistors. Elsevier, Amsterdam.

Benning, M.M., Kuo, J.M., Raushel, F.M., Holden, H.M., 1994. 3-Dimensional structure of phosphotriesterase: an enzyme capable of detoxifying organophosphate nerve agents. Biochemistry 33 (50), 15001–15007.

Caras, S., Janata, J., 1980. Field effect transistor sensitive to penicillin. Anal. Chem. 52, 1935–1937.

Caras, S., Janata, J., 1985. pH based enzyme potentiometric sensors. Parts 1–3. Anal. Chem. 57, 1917–1925.

Chen, K., Li, G., Chen, L., Zhu, Y., 1989. Ion-sensitive field effect transistor with silicon nitride gate for pH sensing. Int. J. Electron. 67, 59–63.

Dave, K.I., Phillips, L., Luckow, V.A., Wild, J.R., 1994. Expression and post-translational processing of a broad spectrum organophosphorous-neurotoxin degrading enzyme in insect tissue culture. Biotechnol. Appl. Biochem. 19, 271–284.

Diot, J.L., Joseph, J., Martin, J.R., Clechet, P., 1985. pH dependence of the Si/SiO_2 interface state density for EOS systems. J. Electroanal. Chem. 193, 75–88.

Dumschat, C., Muller, H., Stein, K., Schwedt, G., 1991. Pesticide sensitive ISFET based on enzyme inhibition. Anal. Chim. Acta 252, 7–9.

Dumas, D.P., Caldwell, S.R., Wild, J.R., Raushel, F.M., 1989. Purification and properties of the phosphotriesterase from *Pseudomonas diminuta*. J. Biol. Chem. 264 (33), 19659–19665.

Dumas, D.P., Durst, H.D., Landis, W.G., Raushel, F.M., Wild, J.R., 1990. Inactivation of organophosphorous nerve agents by the phosphotriesterase from *Pseudomonas diminuta*. Arch. Biochem. Biophys. 277 (1), 155–159.

Dutka, B.J., Nyholm, N., Petersen, J., 1983. Comparison of several microbiological toxicity screening tests. Water. Res. 17, 1363–1368.

Eddowes, M.J., 1985. Response of an enzyme modified pH sensitive ion selective device; consideration of the influence of the buffering capacity of the analyte solution. Sens. Act. 7, 97–115.

Fernando, J.C., Rogers, K.R., Anis, N.A., Valdes, J.J., Thompson, R.G., Eldefrawi, A.T., Eldefrawi, M.E., 1993. Rapid detection of acetylcholinesterase insecticides by a reusable light addressable potentiometric biosensor. J. Agric. Food Chem. 41, 511–516.

Garde, A., Alderman, J., Lane, W., 1995. Development of a pH-sensitive ISFET suitable for fabrication in a volume production environment. Sens. Act. B 27 (1–3), 341–344.

Garde, A., Alderman, J., Lane, W., 1997. Improving the drift and hysteresis of the Si_3N_4 pH response using RTP techniques. Sens. Mater. 9 (1), 15–23.

Grimsley, J.K., Scholtz, J.M., Pace, C.N., Wild, J.R., 1997. Organophosphorus hydrolase is a remarkably stable enzyme that unfolds through a homodimeric intermediate. Biochemistry 36 (47), 14366–14374.

Guilbault, G.G., Tomita, Y., Kolesar, E.S. Jr., 1981. A coated piezoelectric crystal to detect organophosphorous compounds and pesticides. Sens. Act. 2, 43–57.

Hoskin, F.C.G., Kirkish, M.A., Steinmann, K.E., 1984. Two enzymes for the detoxification of organophosphorus compounds-sources, similarities and significance. Fund. Appl. Toxicol. 4, S165–S172.

Janata, J., Huber, R.J., Cohen, R., Kolesar, E.S. Jr., 1981. Chemically sensitive field effect transistor to detect organophosphorus compounds and pesticides. Aviat. Space Environ. Med. 52, 666–671.

Janata, J., Huber, R.J. (Eds.), 1985. Solid State Chemical Sensors. Academic Press, Orlando, FL.

Janata, J., 1994. Twenty years of ion-selective filed effect transistors. Analyst 119, 2275–2278.

Lai, K., Dave, K.I., Wild, J.R., 1994. Bimetallic binding motifs in organophosphorous hydrolase are important for catalysis and structural organization. J. Biol. Chem. 269 (24), 16579–16584.

Lai, K., Grimsley, J.K., Kuhlmann, B.D., Scapozza, L., Harvey, S.P., Defrank, J.J., Kolakowski, J.E., Wild, J.R., 1996. Chimia 50 (9), 430–431.

Lisdat, F., Moritz, W., 1993. A reference element based on a solid state structure. Sens. Act. B 15–16, 228–232.

Lewis, V.E., Donarski, W.J., Wild, J.R., Raushel, F.M., 1988. Mechanism and stereochemical course at phosphorous of the reaction catalyzed by a bacterial phosphotriesterase. Biochemistry 27, 1591–1597.

Makower, A., Barmin, A., Morzunova, T., Eremenko, A., Kuorchkin, I., Bier, F., Scheller, F., 1997. Affinity enzymometric assay for detection of organophosphorus compounds. Anal. Chim. Acta 357 (1–2), 13–20.

McDaniel, C.S., Harper, L.L., Wild, J.R., 1988. J. Bacteriol. 170, 2306–2311.

Noellgen, R.M., Landis, W.G., 1992. Identification and characterization of the organophosphate acid anhydrase activity of the blue mussel, *Mytilus edulis*. Comp. Biochem. Physiol. 101C (3), 615–623.

Nyamsi Hendji, A.M., Jaffrezic-Renault, N., Martelet, C., Clechet, P., Shul'ga, A.A., Strikha, V.I., Netchiporuk, L.I., Soldatkin, A.P., Wlodarski, W.B., 1993. Sensitive detection of pesticides using a differential ISFET-based system with immobilized cholinesterases. Anal. Chim. Acta 281, 3–11.

Pandey, P.C., Weetall, H.H., 1995. An evanescent-wave sensor for the detection of organophosphorous compounds based on the inhibition of cholinesterase. Ind. J. Chem. Technol. 2 (5), 261–265.

Pei, L., Omburo, G., McGuinn, W.D., Petrikovics, I., Dave, K., Raushel, F.M., Wild, J.R., DeLoach, J.R., 1994. Encapsulation of phosphotriesterase within murine erythrocytes. Toxicol. Appl. Pharmacol. 124, 296–301.

Perrot, H., Jaffrezic-Renault, N., De Rooij, N.F., Van Den Vlekkert, H.H., 1989. Sens. Act. 20, 293–299.

Phillips, J.P., Xin, J.H., Kirby, K., Milne, C.P. Jr., Krell, P., Wild, J.R., 1990. Transfer and expression of an organophosphate degrading gene from *Pseudomonas* in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA 87, 8155–8159.

Rainina, E.I., Efremenco, E.N., Varfolomeyev, S.D., Simonian, A.L., Wild, J.R., 1996. The development of a new biosensor based on recombinant *E. coli* for the direct detection of organophosphorus neurotoxins. Biosens. Bioelectron. 11 (10), 991–1000.

Ratner, B.D., Chilkoti, A., Lopez, G.P., 1990. Plasma deposition and treatment for biomaterial applications. In: D'Agostino, R. (Ed.), Plasma deposition, treatment, and etching of polymers. Academic Press, Boston.

Reiner, E., Aldridge, W.N., Hoskin, F.C.G. (Eds.), 1989. Enzymes Hydrolyzing Organophosphorus Compounds. Ellis Horwood, Chichester, UK.

Rogers, K.R., Cao, C.J., Valdes, J.J., Eldefrawi, A.T., Eldefrawi, M.E., 1991. Acetylcholinesterase fiber optic biosensor for detection of anticholinesterases. Fund. Appl. Toxicol. 16, 810–820.

Rocher, V., Chovelon, J.M., Jaffrezic-Renault, N., Cros, Y., Birot, D., 1994. An oxynitride ISFET modified for working in a differential mode for pH detection. J. Electrochem. Soc. 114 (2), 535–539.

Sakai, H., Kaneki, N., Hara, H., 1993. Analytical application for chemicals using an enzyme sensor based on an ISFET. Sens. Act. B 13–14, 578–580.

Shul'ga, A.A., Strikha, V.I., Soldatkin, A.P., El'skaya, A.V., Maupas, H., Martelet, C., Clechet, P., 1993. Removing the influence of buffer concentration on the response of enzyme filed effect transistors by using additional membranes. Anal. Chim. Acta 278, 233–236.

Simonian, A.L., Rainina, E.I., Wild, J.R., 1997. A new approach for discriminative detection of organophosphate neurotoxins in the presence of other cholinesterase inhibitors. Anal. Lett. 30 (4), 2453–2468.

Singh, A.K., Flounders, A.W., Volponi, J.V., Ashley, C.S., Wally, K., Schoeniger, J.S., 1999. Development of sensors for direct detection of organophosphates. Part I: immobilization, characterization and stabilization of acetylcholinesterase and organophosphate hydrolase on silica supports. Biosens. Bioelectron. 14, 703–713.

Strop, P., Guilbault, G.G., 1972. A new assay for cholinesterase potentiometric determinations in flow streams. Anal. Chim. Acta 62, 425–430.

Tahara, S., Yoshii, M., Oka, S., 1982. Electrochemical reference electrode for the ion-selective field effect transistor. Chem Lett. 3, 307–310.

Trojanowicz, M., Hitchman, M.L., 1996. Determination of pesticides using electrochemical biosensors. Trends Anal. Chem. 15 (1), 38–45.

Van Der Schoot, B.H., Bergveld, P., 1987. The pH static enzyme sensor. Anal. Chim. Acta 199, 157–160.

Van Der Schoot, B.H., Bergveld, P., 1990. Evaluation of the sensor properties of the pH static enzyme sensor. Anal. Chim. Acta 233, 49–57.

Van Emon, J.M., Lopez-Avila, V., 1992. Immunochemical methods for environmental analysis. Anal. Chem. 64 (2), 79–88.

Vlasov, Y., Bratov, A., Levichev, S., Tarantov, Y., 1991. Enzyme semiconductor sensor based on butrylcholinesterase. Sens. Act. B 4, 283–286.

Watkins, L.M., Mahoney, H.J., McCulloch, J.K., Raushel, F.M., 1997. Augmented hydrolysis of diisopropylfluorophosphate in engineered mutants of phosphotriesterase. J. Biol. Chem. 272 (41), 25596–25601.

Wong, H.S., White, M.H., 1989. A CMOS integrated ISFET operational amplifier chemical sensor employing differential sensing. IEEE Trans. Electron. Dev. 36 (3), 479–487.

Yang, Q., Atanasov, P., Wilkens, E., Hughes, R.C., 1995. Enzyme electrodes with glucose oxidase immobilized on Stober glass beads. Anal. Lett. 28 (14), 2439–2457.